IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:	Confirmation No.: 6940
DAFTARY, *et al.*	Group Art Unit: 1612
Application Serial No.: 10/748,094	Examiner: Gollamudi KISHORE
Filed: December 31, 2003	Attorney Docket No.: 2912960-001000

For: NON-PEGYLATED LONG-CIRCULATING LIPOSOMES

APPEAL UNDER 35 U.S.C. §134

TABLE OF CONTENTS

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APPEAL BRIEF

I. Real Party in Interest

Bharat Serums & Vaccines, Ltd, by virtue of the assignment executed June 4, 2004, and recorded at reel/frame number 015891/0145 on June 4, 2005, is the real party in interest.

II. Related Appeals and Interferences

Appellants are unaware of any appeals or interferences that will directly affect, be directly affected by, or have a bearing on the present appeal.

III. Status of Claims

Claims 1-8, 10, 12, 14-22, and 63-69 are pending in this application. Claims 1-8, 10, 12, 14-22, and 63-69 are rejected by the November 5, 2009 Final Office Action. Claims 9, 11, 13, and 23-62 are canceled.

IV. Status of Amendments

Following the November 5, 2009 Final Rejection, an Amendment After Final Rejection was filed on February 5, 2010. In response, an Advisory Action was mailed on March 15, 2010 indicating that the claim amendments would not be entered. On March 24, 2010, a Supplemental Amendment After Final Rejection was filed. The amendments therein were entered by the Advisory Action mailed on June 9, 2010. A Notice of Appeal was filed on April 1, 2010.

V. Summary of Claimed Subject Matter

The claims of this application do not stand or fall together. A summary of the claimed subject matter with references to the originally filed specification is outlined below.

Independent claim 1 recites a process for manufacture of long circulating (p. 14, lns. 15-21) non-pegylated liposomes (p. 6, ln. 28-p. 7, ln. 19) comprising: dissolving one or more phospholipids (p. 8, ln. 17-p. 9, ln. 6) and one or more sterols (p. 9, lns. 7-19) in a solvent or mixture of solvents (p. 7, ln. 27-p.8, ln. 5); wherein the one or more phospholipids is a saturated phosphatidylcholine selected from the group consisting of distearoyl phosphatidylcholine (DSPC) (p. 9, ln. 1), hydrogenated soya phosphatidyl-choline (HSPC) (p. 9, ln. 2) and mixtures thereof; removing the solvent or mixture of solvents (p. 7, lns. 25-27; p. 9, lns. 20-22) and adding an aqueous hydration media (p. 9, ln. 22-p.10, ln. 21) to the phospholipids and sterols (p. 7, lns. 25-27); or adding an aqueous hydration media to the phospholipids and sterols in the solution (p. 7, lns. 25-27); and removing the solvent or mixture of solvents (p. 7, lns. 25-27); wherein the aqueous hydration media comprises ammonium sulfate (p. 9, lns. 28-29; p. 12, lns. 1-15) and sucrose (p. 9, ln. 29-p. 10, ln. 3; p. 11, lns. 26-33; p. 12, lns. 1-15) and the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present (p.

10, lns. 16-18) to form long circulating non-pegylated liposomes; and removing ammonium sulphate from extraliposomal hydration medium by dialysis (p. 10, ln. 33-p. 11, ln. 6; p. 12, lns. 16-18) using a sucrose-histidine buffer solution (p. 12, lns. 19-31).

Independent claim 63 recites a process for manufacture of non-pegylated (p. 6, ln. 28-p. 7, ln. 19) liposomes comprising: forming a lipid film by evaporating a solvent from a lipid solution (p. 7, lns. 25-27) comprising one or more phospholipids (p. 8, ln. 17-p. 9, ln. 6), a sterol (p. 9, lns. 7-19), and a solvent (p. 7, ln. 27-p.8, ln. 5); and hydrating the lipid film (p. 7, lns. 25-27) by adding an aqueous hydration media (p. 9, ln. 22-p.10, ln. 21) to form a non-pegylated liposomal composition; wherein the aqueous hydration media comprises ammonium sulfate (p. 9, lns. 28-29; p. 12, lns. 1-15) and sucrose (p. 9, ln. 29-p. 10, ln. 3; p. 11, lns. 26-33; p. 12, lns. 1-15) and wherein the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present in the lipid solution (p. 10, lns. 16-18); and removing ammonium sulphate from extraliposomal hydration medium (p. 10, ln. 33-p. 11, ln. 6; p. 12, lns. 16-18) using a sucrose-histidine buffer solution (p. 12, lns. 19-31).

VI. Grounds of Rejection to be Reviewed on Appeal

1. Whether claims 1-8, 10, 12, 14-22, and 63-69 comply with the written description requirement under 35 U.S.C. §112, first paragraph.
2. Whether claim 69 is indefinite under 35 U.S.C. §112, second paragraph.
3. Whether claims 1-8, 10, 12, 14-22, and 63-69 are unpatentable under 35 U.S.C. §103(a) over U.S. Patent No. 6,110,491 to Kirpotin ("Kirpotin") in view of U.S. Patent Application Publication No. 2005/0025822 to Wong et al. ("Wong") and U.S. Patent Application Publication No. 2006/0078605 to Mammarella ("Mammarella")

individually or in further combination with U.S. Patent No. 4,235,871 to Papahadjopoulos et al. ("Papahadjopoulos").

4. Whether claims 1-8, 10, 12, 14-22, and 63-69 are unpatentable under 35 U.S.C. §103(a) over Hong et al., *Direct Comparison of Liposomal Doxorubicin with or without Polyethylene Glycol Coating in C-26 Tumor-bearing Mice: Is surface coating with polyethylene glycol beneficial?*, 5 Clinical Cancer Research 3645-3652 (1999) ("Hong") in view of Wong and Mammarella individually or in combination with Papahadjopoulos and U.S. Patent No. 4,880,635 to Janoff et al. ("Janoff").
5. Whether claims 1-8, 10, 12, 14-22, and 63-69 are unpatentable under 35 U.S.C. §103(a) over Hong in view of Wong and Mammarella individually or in combination with Papahadjopoulos and Janoff, and either U.S. Patent No. 5,192,528 to Radhakrishnan et al. ("Radhakrishnan") or Uchiyama et al., *Effects of the size and fluidity of liposomes on their accumulation in tumors: A presumption of there interaction with tumors*, 121 International Journal of Pharmaceutics 195-203 (1995) ("Uchiyama").
6. Whether claims 1-8, 10, 12, 14-22, and 63-69 are unpatentable under 35 U.S.C. §103(a) over U.S. Patent No. 5,714,163 to Forssen et al. ("Forssen") in view of Wong and Mammarella.
7. Whether claims 1-8, 10, 12, 14-22, and 63-69 are unpatentable under 35 U.S.C. §103(a) over Forssen in view of Wong and Mammarella individually or in further combination with either Radhakrishnan, Uchiyama, Papahadjopoulos, or Janoff.

8. Whether claims 1-8, 10, 12, 14-22, and 63-69 are unpatentable under 35 U.S.C. §103(a) over Forssen in view of Wong and Mammarella individually or in combination with either Radhakrishnan or Uchiyama and further in view of Kirpotin.

VII. Arguments

A. §112 Rejectionss

1. The Claims Comply With The Written Description Requirement

The Examiner rejects claims 1-8, 10, 12, 14-22, and 63-69 under 35 U.S.C. §112, first paragraph, asserting that "[t]here is no support for the limitation, 'removing ammonium sulphate from extraliposomal hydration medium by dialysis, ultra filtration or column chromatography using a sucrose-histidine buffer solution.'" Final Office Action at page 2. However, after entry of the March 24, 2010 Supplemental Amendment by the June 9, 2010 Advisory Action, claim 1 now recites: "removing ammonium sulphate from extraliposomal hydration medium by dialysis using a sucrose-histidine buffer solution."

Support for the phrase "removing ammonium sulphate from extraliposomal hydration medium by dialysis using a sucrose-histidine buffer solution" may be found in the Specification, for example, at page 4, line 25 – page 5, line 6 and page 40, lines 12 – 18. Specifically, as set forth in the Specification at page 40, lines 12 – 18:

> The suspension of the sized liposomes was dialyzed against a histidine buffer. A tangential flow filtration system was used for the dialysis. The dialysis was continued till extra liposomal ammonium sulfate was removed. The absence of ammonium sulfate in extra liposomal media was confirmed using Nesseler reagent. The histidine hydrochloride solution used in the dialysis and drug loading (below) was as follows: 170.0 gm of sucrose, 3.40 gm of histidine HCl, 1.7 Liters of water, and sodium hydroxide at a quantity sufficient to adjust pH to 6.0 to 6.5.

As set forth above, the claims are supported by the Specification and therefore satisfy the written description requirement under 35 U.S.C. §112, first paragraph. Accordingly, Appellants respectfully request withdrawal of this ground of rejection.

2. Claim 69 Is Not Indefinite

The Examiner rejects claim 69 under 35 U.S.C. §112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Final Office Action at page 3. In rejecting the claim, the Examiner asserts that it is unclear how one can replace ammonium sulfate with a sucrose-histidine solution in an ultra-filtration process. *Id.* Appellants respectfully disagree, but have amended claim 1 solely to facilitate prosecution by removing the term "ultrafiltration." Accordingly, Appellants submit that the pending claim rejection is hereby rendered moot.

Moreover, in rejecting the claims, the Examiner asserts that "[i]t is unclear as to what applicant intends to convey by at least 25 times longer than conventional ***non-liposomal*** formulations when tested in Swiss albino mice at equivalent doses." Office Action at page 3. (Emphasis in original). Appellants disagree and respectfully submit that the scope of claim 69 is clear. That is, the phrase "long circulating non-pegylated liposomes have a blood circulation half life of at least 25 times longer than conventional non-liposomal formulations when tested in Swiss albino mice at equivalent doses" is clearly supported and defined by the Specification. *See* Specification, for example, at page 5, lines 28-30. Further, one skilled in the art would understand how to test the blood circulation half life and any pharmaceutical. Further the term, "conventional non-liposomal formulations" is not unclear as the specification even provides a specific example – ADRIAMYCIN- as a conventional non-liposomal formulation of doxorubicin. Further, the specification contains numerous examples where liposomes of the present invention were compared against ADRIAMYCIN (the marketed conventional preparation of doxorubicin). *See, e.g.*, Examples 2, 3, 4, 5 and 6 showing administration to mice

liposomes of the present invention and ADRIAMYCIN and testing for LD50, sub-acute toxicity, pharmacokinetics, and maximum tolerated dose.

As set forth in M.P.E.P. §2173.04, "[b]readth of a claim is not to be equated with indefiniteness. *In re Miller*, 441 F.2d 689, 169 USPQ 597 (CCPA 1971). If the scope of the subject matter embraced by the claims is clear, and if applicants have not otherwise indicated that they intend the invention to be of a scope different from that defined in the claims, then the claims comply with 35 U.S.C. 112, second paragraph." Because the scope of claim 69 is clear and unambiguously defined by the Specification, claim 69 satisfies 35 U.S.C. 112, second paragraph. Accordingly, applicants respectfully request withdrawal of this ground of rejection.

B. §103(a) Rejections

The Examiner has relied on a combination of nine references for the various 35 U.S.C. §103(a) rejections. As has been argued during prosecution and shown again herein, none of the cited references, alone or in combination, teach each and every element of the claims. In fact, as shown herein below, in some cases, the references actually teach away from using the recited element. Applicants respectfully submit that the Examiner has appeared to pick and choose certain words from the cited references to argue they teach an element of the claim, and in so doing, has ignored how the allegedly-taught element has actually been used in the reference. Appellants have attached a claim chart at §IX at Appendix 2 that summarizes the alleged teachings of the cited art against the elements of claim 1. This chart summarizes the arguments and clearly shows that the combination of all of these references fails to teach or suggest each and every claim element. Among numerous deficiencies in both the references' teachings and the Examiner's reasons for combining the references, the main two elements of the

claims not taught or suggested by the references are: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution. Accordingly, Appellants request withdrawal of all the grounds of rejection for all of the pending claims and request a Notice of Allowance.

1. Wong And Mammarella Are Not Prior Art

Claims 1-8, 10, 12, 14-22, and 63-69 are rejected under 35 U.S.C. 103(a) as being unpatentable over six varied combinations of the following nine references: Kirpotin, Wong, Mammarella, Papahadjopoulos, Hong, Janoff, Radhakrishnan, Uchiyama, and Forssen. In each of the six combinations, the examiner has relied on Wong and Mammarella. All of the applied reference combinations (and rejections) are fatally flawed by their application of Wong and Mammarella because neither Wong nor Mammarella is proper prior art in that the instant application.

The instant application is entitled to a priority date of at least December 31, 2002. A proper priority claim was made to Provisional Patent Application No. 1101/Mum/02, filed on December 31, 2002, and this claim has been previously perfected by the submission of a Certified English-language copy of Provisional Patent Application No. 1101/Mum/02, submitted to the USPTO on April 18, 2007.

a. Wong (US 2005/0025822) Is Not Prior Art

Wong is not proper prior art against the claims. Wong was filed on May 24, 2004 and claims priority to U.S. Provisional Application No. 60/475,080, filed on May 30, 2003. The

November 5, 2009 Office Action states on page 14 that the priority date of Wong is May 30, 2003. This date is after the effective December 31, 2002 priority date of the instant application and, as such, Wong cannot be applied as prior art under 35 U.S.C. §103(a).

b. Mammarella (US 2006/0078605) Is Not Prior Art

Mammarella is not proper prior art against the claims. Mammarella has a publication date of April 16, 2006. The November 5, 2009 Office Action states on page 14 that Mammarella has a priority date of August 28, 2003. This date is after the effective December 31, 2002 priority date of the instant application and, as such, Mammarella cannot be applied as prior art under 35 U.S.C. §103(a). At least because Wong and Mammarella are not proper prior art under 35 U.S.C. §103(a), each of the applied reference combinations is deficient. Accordingly, Appellants respectfully request withdrawal of the obviousness rejections.

2. The References Fail To Teach Or Suggest An Aqueous Hydration Buffer Comprising Ammonium Sulfate And Sucrose

None of the cited references, alone or in combination, teach or suggest "wherein the aqueous hydration buffer comprises ammonium sulfate and sucrose" as recited in independent claims 1 and 63.

Kirpotin does not teach an aqueous hydration buffer comprising ammonium sulfate and sucrose. Kirpotin actually teaches away from using ammonium sulfate and sucrose because when Kirpotin used ammonium sulfate alone, his pegylated liposomes had the lowest performance. Kirpotin found that ammonium sulfate gives a poor precipitation and thus poor loading efficiency but found that polyacrylate type precipitating agents in the hydration medium

provided a better loading efficiency. One skilled in the art after reading Kirpotin would thus not be motivated to take ammonium sulfate, which did not work well, and combine it with sucrose when Kirpotin leads the reader to abandon the use of ammonium sulfate altogether and instead use a polymer such as polyacrylate. Further, Kirpotin is directed to a loading mechanism and this mechanism involves forming a precipitate with a polymer, whereas the ammonium sulfate and sucrose solution used in the present invention is for forming the liposomes. There is thus no teaching or suggestion by Kirpotin to use a hydration buffer comprising ammonium sulfate and sucrose, let alone combine it with any other reference teaching ammonium sulfate or sucrose.

In addition, Papahadjopoulos, Hong, Radhakrishnan, and Uchiyama do not use ammonium sulfate and sucrose in the hydration buffer either. Further, Janoff does not teach a hydration buffer comprising ammonium sulfate and sucrose. Moreover, Janoff does not even teach hydration of phospholipids to prepare liposomes; instead it teaches hydrating already formed liposomes, for their stability in dehydration.

Forssen also does not teach an aqueous hydration buffer comprising ammonium sulfate and sucrose. Forssen has a buffer with ammonium salt or sucrose but does not teach a hydration buffer with ammonium sulfate and sucrose.

Wong and Mammarella did not use ammonium sulfate and sucrose and in addition, these references are not proper prior art as they are after the priority date of the present application.

Thus, none of the cited art, alone or in combination, teaches or suggests the claim element of an aqueous hydration media comprising ammonium sulfate and sucrose. For this reason alone, all of the 35 U.S.C. §103(a) rejections should be withdrawn.

3. The References Fail To Teach Or Suggest Removing Extraliposomal Hydration Media Using A Sucrose-Histidine Buffer Solution

None of the cited references, alone or in combination, teach or suggest "removing ammonium sulphate from extraliposomal hydration medium using a sucrose-histidine buffer solution" as recited in claim 63 or doing the same "by dialysis" as recited in claim 1.

Neither Kirpotin, Forssen, Janoff, Papahadjopoulos, Hong, Radhakrishnan, Uchiyama, Mammarella, nor Wong teach or suggest removing extraliposomal hydration media using a sucrose-histidine buffer solution, as included by the claims. In addition, as mentioned above, Mammarella and Wong are not proper prior art references. Although Papahadjopoulos mentions histidine in a hydration buffer, there is no teaching or suggestion to use a sucrose-histidine buffer solution for removing extraliposomal hydration media.

Thus, none of the cited art teaches or suggests the claim element of removing extraliposomal hydration media using a sucrose-histidine buffer solution. For this reason alone, all of the 35 U.S.C. §103(a) rejections should be withdrawn.

4. The Reference Combinations Are Fatally Flawed With Respect To Claims 1 and 63

The nine applied references are asserted in various combinations. The above-described deficiencies (see §§B.1-3) equally apply to all of the applied combinations. These deficiencies alone are sufficient to withdraw the current rejections and allow the current claims.

However, in addition to these ubiquitous failures, the applied reference combinations have further failures that are more specific to each specific applied combination and the

references therein. These deficiencies further evidence the non-obviousness of the instant claims over the varied and unreasonable applied reference combinations.

These deficiencies are organized by reference, as opposed to asserted combination, to avoid repetition because each of the references is applied in at least two reference combinations. §§VII.B.4.a-c address the primary references. §§VII.B.4.d-g address the secondary and tertiary references and their failure to cure the varied deficiencies of the primary references.

a. Deficiencies Specific To Kirpotin (US 6,110,491)

Kirpotin is applied in grounds 3 and 8. In each of these combinations, Kirpotin is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. Kirpotin does not teach or suggest, *inter alia*, at least two elements of the claims: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

(1) Kirpotin Teaches Away From Claims 1 and 63

Kirpotin teaches away from the instant claims. The Final Rejection states on page 4 that:

> Kirpotin discloses a method of preparation of liposomes forming lipid film and hydrating it with a buffer containing ammonium sulphate (Example 7). Kirpotin also teaches that if necessary, to achieve an osmolarity of 377 mmole/kg, sucrose could be added to the medium (Example 8). The liposomes contain hydrogenated egg phospholipid and cholesterol. Doxorubicin is loaded into the preformed liposomes (Example 7). Although in the examples Kirpotin uses PEG-phospholipids, on col. 9 lines 22-23 teaches either the naturally occurring or synthetic phospholipids which implies that use of PEG phospholipids for the method of preparations is not necessary. What is lacking in Kirpotin is the

teaching of the amount of aqueous medium added to per mol of phospholipid.

First, Appellants respectfully note that Kirpotin is concerned with liposome loading and does not teach anything about a process for making a long-circulating non-pegylated liposome.

Second, Example 7 of Kirpotin teaches that loading of the liposomes "containing ammonium sulfate in the absence of ammonium ion gradient absorbed very small amounts of doxorubicin." *See* col. 14, lines 63-65. Further, Kirpotin states: "while the liposomes containing ammonium polyacrylate absorbed considerable amounts of doxorubicin even without ammonium ion gradient." *See* col. 14, lines 65-67. Thus, this example teaches away from using ammonium sulfate in the loading buffer because ammonium polyacrylate provided a much better loading efficiency. The reference teaches that ammonium polyacrylate works much better than ammonium sulphate in liposome loading. Further, Example 7 uses ammonium sulphate inside the liposomes via the hydration medium but it says: "[a]t this temperature, doxorubicin did not form a detectable precipitate in the presence of sulfate anion, but was visibly precipitated by a polyacrylate anion," which again teaches away from the use of ammonium sulphate. *See* col. 14, 40-42.

Thus, Example 7 shows that the use of ammonium sulphate in the hydrating medium in a pegylated (PEG-DSPE containing) liposome is not useful for entrapping drug in the desired amounts. Appellants submit that the Office Action has not provided an explanation as to how this in any way teaches, suggests, or motivates one skilled in the art to use ammonium sulphate in a hydration medium for hydrating non-pegylated phospholipids. Further, the phospholipids used in this example are DSPE, which are clearly not the phospholipids used or claimed in the instant invention (the claims recite the use of only three "phosphatidylcholines," which do not

include DSPE). Further, claim 1 does not contain hydrogenated egg phosphatidyl choline as used in Examples 7 and 8.

Third, in Example 8 of Kirpotin, the phospholipids contain PEG-DSPE and egg phosphatidyl choline. For one of skill in the art, consideration of hydration media containing HEPES buffer and restricted by osmolarity is not relevant when the phospholipids used are outside the scope of claim 1. In Example 8, the hydration buffer can be made with many different salts and of all the salts, the patent shows that the use of ammonium sulphate results in low entrapment performance, compared to others—the best performance being that of polyacrylate. Thus, appellants assert that a person skilled in the art would not choose ammonium sulphate out of all the materials listed in Kirpotin, when its performance is worse than the other materials such as ammonium polyacrylate. Kirpotin indicates a method that does not prefer ammonium ion, and additionally Kirpotin contraindicates the presence of ammonium ion in the hydration media. There would be no reason or motivation to use ammonium ions or ammonium sulphate in the hydration media when such use of ammonium sulphate has not been shown to be of any advantage, and in fact has shown a disadvantage over the use of polyacrylate.

Fourth, Example 9 points out that the amount of doxorubicin hydrochloride precipitating in the inner medium is higher when polyacrylate is used as compared to sulphate. This fact also discourages the use of sulphate against polyacrylate and other anions such as citrate, phosphate, DTPA, which were shown to work better for liposome loading.

The Examiner provides no explanation as to why one skilled in the art after reading Kirpotin would select the choice of hydration medium or inner buffer as ammonium sulphate and sucrose when the reference teaches that sulphate is an inferior anion for liposome loading as compared to the other recited anions. In addition, there is no explanation as to how one skilled in

the art from reading Kirpotin would arrive at the use of ammonium sulfate and sucrose in a hydration buffer in the claimed amounts to achieve long-circulating liposomes. Further, the Examiner has provided no reason why one skilled in the art would expect that this use of ammonium sulphate would provide liposomes with a long-circulation time as required by the claims of the instant invention.

Similar data from Kirpotin's Example 1 together with Example 4 also further support the above arguments. Example 5 also shows superiority of polyphosphate over sodium sulphate. Kirpotin's teachings clearly discourage the use of ammonium sulphate in a hydration media for hydration of phospholipids, as Kirpotin teaches that polymers are better than ammonium/ sodium sulphate/sulphate anion. Thus, on the one hand, the Examiner points to Kirpotin for non-pegylated liposomes, and on the other hand, the Examiner points to Examples 7 and 8 which involve pegylated liposomes. The Examiner has pointed to nothing that explains why the teaching in Kirpotin would have led the skilled reader to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for hydrating phospholipid would reduce leakage from a loaded liposome and provide a long-circulating liposome that does not contain PEG, nor do the variously applied references cure such critical deficiencies.

(2) Kirpotin does not teach or suggest the use of sucrose in the aqueous hydration media as required by the claims

As described above, Kirpotin does not teach the use of sucrose in the aqueous hydration media. There would be no reason or motivation to use sucrose in the aqueous hydration media as claimed in the present invention in Kirpotin In column 5, 9-21, Kirpotin discusses the liposome composition and in particular discusses the charged-polymer precipitating agent:

1. Acidic and basic polysaccharides, both natural and natural-derived, including: polygalacturonates, hyaluronic acid, gum arabic, chondroitin sulfates A, B, and C, keratin sulfates, dermatan sulfates, heparin and its derivatives, pectin and its derivatives, alginic (poly-anhydromannuronic) acid, teichoic acids, chitosans; derivatives of cellulose, amylose, amylopectin, dextran, or other neutral polysaccharide obtained by introduction of carboxyalkyl, phosphate, sulphate, amino-, mono-, di-, trialkylamino, tetraalkylammonium functional groups, derivatives of the said polysaccharides with nitrogen heterocycles, and derivatives obtained by grafting other ionizable functions to polysaccharide backbone.

This list clearly shows that the use of sucrose or neutral saccharide without any charge in the hydration medium is not taught or suggested and, in fact, is ruled-out. Only derivatives of saccharides are considered as precipitating agents for inclusion in the hydration media. The presence of any other material inside the liposome is not suggested. Thus, there is simply no teaching or reason for adding sucrose in the hydration media arising out of Kirpotin.

(3) Kirpotin does not teach or suggest removal of the organic solvent after hydration of the liposomes, which is an element of the present claimed invention

Another element in the claims that is not present in Kirpotin relates to removal of the organic solvent. As pointed out above, Kirpotin's method involves removal of the organic solvent before the hydration. The present claims recite that the solvent can be removed before or after hydrating the lipids and sterols. Nowhere does Kirpotin teach or suggest that the organic solvent could be removed after hydration.

(4) Kirpotin does not teach or suggest a method of making long-circulating non-pegylated liposomes

The Final Rejection overreaches when it asserts that Kirpotin's mention of using synthetic or naturally occurring phospholipids implies that the use of Peg-phospholipids is not necessary. First, synthetic or naturally occurring phospholipids could be pegylated – there is nothing to indicate that they are not pegylated, especially given the fact that every single example in Kirpotin uses pegylated phospholipids. Second, Kirpotin relates to loading liposomes—so why would one skilled in the art take a reference in which every method of loading a liposome discussed therein uses pegylated phospholipids to arrive at the claimed invention, which does not use pegylated phospholipids (and which further contains other claim limitations not even taught or suggested by Kirpotin)? For instance, the present invention relates to a method of making long-circulating liposomes that expressly do not contain pegylated phospholipids, and in addition, the claimed method employs various specific steps and reagents (also not taught or suggested by Kirpotin) to achieve the long-circulating liposomes. The Examiner has not pointed out why one skilled in the art would even look to Kirpotin at all to achieve long-circulating liposomes when Kirpotin does not even address or mention this issue.

(5) Unexpected results are unnecessary to distinguish the claims over Kirpotin in view of the variously applied references

The deficiencies of Kirpotin are substantial and clear. However, the Examiner states on page 5 that: "However and since complete hydration of the phospholipid is required for the formation of liposomes, in the absence of showing unexpected results, it is deemed obvious to

one of ordinary skill in the art to vary the amounts of the hydrating medium to obtain the best possible results."

Appellants respectfully point out that the liposome product of Kirpotin is not the same as the claimed product because it gives rise to hand foot syndrome on usage (because of the presence of pegylated phospholipids). Undeniably, all of the liposomes made in Kirpotin are pegylated liposomes, whereas as per claim 1 of the instant invention the product produced by the method is a "non-pegylated liposome." Even accepting the Examiner's argument that the mention of synthetic and naturally occurring phospholipids suggests non-pegylated liposomes, it clearly does not teach or suggest a composition comprising only non-pegylated liposomes.

The difference between Kirpotin and the present invention is not simply "varying the amounts of hydrating medium to obtain the best possible results," as asserted. First of all, what are the "best possible results" to which the Office action refers? Kirpotin relates to liposome loading, so, arguably the "best possible results" relate to obtaining a high loading efficiency. In contrast, the present invention relates to obtaining a long-circulating liposome without the use of PEG so arguably the "best possible results" relate to achieving a long lasting stable liposome not using pegylated phospholipids. Where is the argument or evidence to show that it would be obvious to look for what is lacking in Kirpotin relating to liposome loading to combine with another reference relating to entrapment quality of the liposomes (i.e. Papahadjopoulos discussed below) to create a long-circulating liposome that does not contain PEG?

The Appellants submitted a Declaration (*see* §IX at Appendix 1) showing unexpected results comparing liposomes made by the present invention against liposomes containing pegylated phospholipids (Caelyx). The Examiner has refused to consider these unexpected results and has indicated that the unexpected results need to arrive from the comparison of

Kirpotin and liposomes made by the present invention. Since the Examiner states that the liposomes made by the current method should be compared with the Kirpotin product, could the Examiner define the Kirpotin product to be used for comparison? All examples in Kirpotin are pegylated (so comparison of Caelyx, which is a pegylated liposome, should suffice). Further, which hydration media should be used in the non-pegylated composition that is characteristic of Kirpotin? Which method of loading is to be used—co-precipitation with charged precipitating agent or in the form of pH induced precipitate? Which of the various polymers described in Kirpotin should be used in the hydration media? In other words, which of the numerous methods and liposome composition possibilities mentioned in Kirpotin should be used in a comparison?

There is nothing in the law that states that a claimed product has to be compared to the prior art cited by the Office Action. An applicant relying upon a comparative showing to rebut a *prima facie* case of obviousness may compare his claimed invention with the closest prior art (and not necessarily the art cited by the Examiner). *See Application of Merchant*, 575 F.2d 865, 869 (CCPA 1978). In this case, the Board rejected an application comparing the claimed invention against a prior art reference and not against the prior art reference cited by the Examiner. The Examiner/Board erroneously required the applicant to provide unexpected results over the Examiner's cited art. The CCPA noted that this "approach lacks a basis in law. To apply that approach would place a burden upon the applicant to provide comparison tests of his invention with every cited reference, for each reference may be said to be the "closest" prior art for the particular limitation it allegedly discloses." Appellants respectfully assert that the closest prior art is the liposome product Caelyx (see further discussion of Caelyx below) and as such the Declaration should be considered as it provides the comparison of liposomes made by the claimed method to the closest prior art. Appellants have taken one of the main differences from

the prior art (pegylated liposomes) and the claimed invention (non-pegylated) liposomes and compared their circulation times to show that the claimed invention indeed has a long-circulation time and is just as, if not more effective than the prior art pegylated liposomes (Caelyx).

These multiple deficiencies are not cured by the applied references that are applied along with Kirpotin in grounds 3 and 8. Indeed, the references that are asserted to cure the acknowledged deficiencies of Kirpotin are themselves deficient for the reasons presented below.

b. Deficiencies Specific To Hong (5 Clinical Cancer Research 3645-3652)(1999)

Hong is applied in grounds 4 and 5. In each of these combinations, Hong is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. Hong does not teach or suggest, *inter alia*, at least two elements of the claims: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

First, Hong teaches using pegylated liposomes to prolong liposome circulation. Thus, the product of Hong results in the problems that the instant inventions solves, such as Hand and Foot Syndrome.

Additionally, there is no teaching in Hong that would have led one of skill in the art to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for hydrating phospholipid would reduce leakage from a loaded liposome. Further, there is no evidence to say that leakage of liposomes has any relation with long circulation of liposomes, as required by the claims.

Among other things, Hong demonstrates that there is not a "standard" pegylated liposome. The pharmaceutical properties of pegylated or non-pegylated liposomes vary from preparation to preparation. Amongst the pegylated liposomes the extent of pegylation, the molecular weight of PEG used, the manner of pegylation make a difference, and there is no point in insisting that the comparison of the product of the instant invention should be made with any of the Kirpotin liposomes. The internal comparison with the comparative example wherein most of the steps and materials are the same is the best comparison. This has been done. *See* Declaration at §IX. Appendix 1. Comparison with references, such as Kirpotin, Forssen, and Hong is unproductive, especially when the products of these asserted primary references differs so greatly from that of the claims.

These deficiencies are not cured by the applied references that are applied along with Hong in grounds 2 and 3. Indeed, the references that are asserted to cure the acknowledged deficiencies of Hong are themselves deficient for the reasons presented above and below.

c. Deficiencies Specific to Forssen (US 5,714,163)

Forssen is applied in grounds 6, 7, and 8. In each of these combinations, Forssen is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. As stated above, Forssen does not teach or suggest, *inter alia*, at least two elements of the claims: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution. A detailed analysis follows:

(1) Forssen does not teach or suggest the claimed hydration medium

In Example 1 of Forssen, a spray-dried distearoylphosphatidylcholine/cholesterol (DSPC/CHOL) lipid was hydrated with either a buffer containing the ammonium salt of one of the counter-ions or 300 mM sucrose. One of the 10 buffers used contained ammonium sulfate. After sonication, annealing, centrifuging and buffer exchange by gel filtration on a Sephadex column previously equilibrated with unbuffered 300 mM sucrose, vincristine was ion-exchange loaded by incubation of the liposomes with an aqueous solution of its sulfate salt.

Forssen relates to phosphatidyl choline/cholesterol (PC/CHOL) liposomes containing vincristine or other cationic vinca alkaloids and an anion in an aqueous phase of liposome. Reference is made to several prior art methods for forming liposomes and there is no suggestion that Forssen teaches any new or modified method of liposome formation. It is stated that a significant benefit of the liposomes used by Forssen is that they can be prepared without a transmembrane or pH gradient (*see* column 4, lines 26-30). In the generally exemplified process, PC/CHOL is hydrated with aqueous anion and the vinca alkaloid is loaded by ion-exchange loading (*see* column 4, line 49 to column 5, line 10).

Column 6, lines 22-25 describe what can be used in the buffer in the Forssen process: "Vesicles were prepared by hydrating approximately 500 mg of sprayed-dried lipids, DSPC:Cholesterol (2:1, mole ratio), at 65°C with either a buffer containing the ammonium salt of one of the counter-ions or 300 mM sucrose" (emphasis added).

Appellants direct the Board to Table 1 of Example 1 of Forssen (col. 6, line 53 to col.7 line - 14). When the counter ion is tartrate, the percent of vincristin entrapped is 90% and when the counter ion is sulphate, the percent of vincristin entrapped drops to 49%. This table shows

that different counter-ions have different entrapment rates. The Examiner has not explained why one skilled in the art looking at Example 1 of Forssen would choose sulphate as a counter ion especially when there are other ions (except succinate) giving entrapment above 67%. Further, Table 1 has no data for vincristine entrapment when "sucrose" is used (not along with counter ion). Thus, there is no teaching here of a buffer comprising ammonium sulphate and sucrose.

If one reads the same example completely, further down in the animal experiment there is a sentence in column 7, lines 26-32 that reads as follows:

> The mice were randomized into 11 treatment groups and therapy was initiated four days after tumor implantation. The chemotherapeutic treatment groups consisted of free Vcr and nine vesicle-Vcr formulations (Vcr salts of glutamate, tartrate, hydrogen diphosphate, aspartate, EDTA, succinate, pyrophosphate, lactobionate, and citrate). Dosing was at 2.5 mg/kg. Tumor-bearing untreated controls received a treatment of 300 mM sucrose in a volume equivalent to the experimental groups.

Notably from this passage, sulphate is not at all used, which clearly shows that the inventors of Forssen did not think sulphate was useful.

Thus, there is no teaching in Forssen that would have led one of ordinary skill in the art to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for hydrating phospholipid would reduce leakage from a loaded liposome. Moreover, there is no reason to conclude from Forssen that sucrose would be required for decreasing leakage or prolonging circulation and/or increasing efficiency in reduction in breast tumor if doxorubicin is loaded by the process of the present invention.

Further, the fact that sucrose or other sugars inhibit leakage of an encapsulated active material from dehydrated liposomes is no indication that it will prevent leakage from the

liposomes prior to dehydration or from liposomes that need no dehydration or from the rehydrated liposomes after loading with the required drug or other active material.

(2) Forssen does not teach or suggest the claimed sucrose-histidine buffer

Moreover, Forssen does not use sucrose-histidine buffer for removing extraliposomal hydration salt. Using a sucrose-histidine buffer for washing extraliposomal hydration salts (ammonium sulphate) is an ingenious technique of getting sucrose in the outside layer. This feature is not an obvious process step, especially in view of the deficient art, and by itself has inventive merit, in achieving removal of hydration media salts and depositing sucrose on the outside layer of the liposome after the liposomes are sized.

These deficiencies are not cured by the applied references that are applied along with Forssen in grounds 6, 7, and 8. Indeed, the references that are asserted to cure the acknowledged deficiencies of Kirpotin are themselves deficient for the reasons presented above and below.

d. Deficiencies Specific to Wong (US 2005/0025822)

Wong is applied in grounds 3-8. The Examiner has cited Wong as allegedly teaching the use of a sucrose-histidine buffer to remove extra-liposomal ammonium sulphate for dialysis after hydration of the phospholipids and liposomes are formed. Wong does not teach or suggest, *inter alia*, at least two elements of the claims: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution. Further, as described above, Wong is NOT proper prior art. However, even if it was, it would still be deficient in its asserted teachings and uncured by the remaining references for at least the following reasons.

(1) Wong does not teach or suggest non-pegylated liposomes

Wong intends to prepare liposomes and aerosolize them for delivery to the lung. Wong states at [0031]:

> Another lipid component in the liposomes of the present invention, is a vesicle-forming lipid derivatized with a hydrophilic polymer. This lipopolymer component results in formation of a liposome surface coating with hydrophilic polymer chains on both the inner and outer lipid bilayer surfaces. Typically, between about 1-20 mole percent of the lipopolymer is included in the lipid composition. Liposomes having a surface coating of hydrophilic polymer chains, such as polyethylene glycol (PEG), are desirable as drug carries as these liposomes offer an extended blood circulation lifetime over liposomes lacking the polymer coating. The polymer acts as a barrier to blood proteins thereby preventing binding of the protein and recognition of the liposomes for uptake and removal by macrophages and other cells of the reticuloendothelial system

(emphasis added). As disclosed in [0033], a preferred lipopolymer is mPEG-DPSE.

Thus, Wong intends to extend the blood circulation time by using pegylated phospholipids, whereas the present invention is for extending blood circulation time with non-pegylated liposomes. Though Wong's process can be used for making non-pegylated liposomes, according to Wong, these would not extended blood circulation life time, which is required by the present claims. For at least these reasons, one of ordinary skill would not have combined Wong with the applied references to yield the instant claims.

Furthermore, Wong clearly show that one skilled in the art even after the filing date of the present invention believed that non-pegylated liposomes would provide an extended blood circulation life time.

(2) **Wong does not teach or suggest "removing ammonium sulphate from extraliposomal hydration medium by dialysis using a sucrose-histidine buffer solution"**

As with all the other references, Wong does not teach or suggest using a sucrose-histidine buffer solution as claimed. The Examples of Wong clearly reveal this deficiency.

For instance, Example 1 (paragraph [0063]) states:

> HSPC, cholesterol, and, in some formulations, mPEG-DSPE were solubilized in ethanol. Multilamellar vesicles were formed using the ethanol injection technique where the ethanol solution of lipids were hydrated in ammonium sulfate at pH 5.5 and at 65°C. Liposomes were downsized to ~150 nm by extrusion through an extruder at 65°C. using serial size decreasing membranes--0.4 μm, 0.2 μm and 0.1 μm. External ammonium sulfate was removed by exchanging against 10% **sucrose**, NaCl (pH=5.5) using diafiltration to generate an ion gradient. Ciprofloxacin was solubilized in 10% sucrose and incubated with the liposomes at 65°C for 30-60 min. Free ciprofloxacin was removed using diafiltration against 10% sucrose, NaCl. Typical loading resulted in 40-60% of initial drug concentration loaded into liposomes. The final solution was in a 10 mM histidine and 10% sucrose buffer. Typical drug to lipid ratios were 0.3-0.5 (w/w)

(emphasis added). Thus, external ammonium sulfate was removed by exchanging against 10% sucrose, NaCl (pH=5.5) using diafiltration to generate an ion gradient. There is no histidine used in this diafiltration buffer, whereas the present invention uses sucrose-histidine in the dialysis buffer for removing extraliposomal ammonium sulphate. Keeping the liposomes finally in a 10 mM histidine and 10% sucrose buffer in no way indicates or suggests the use of a sucrose-histidine buffer in the dialysis step.

For at least these reasons, one of skill in the art would not have combined the teachings of Wong with the other cited references and even if such combinations were made, they would still be deficient in teaching or suggesting the features of the claims.

e. Deficiencies Specific to Mammarella (US 2006/0078605)

Mammarella is applied in grounds 3-8. The Examiner has cited Mammarella as allegedly teaching the use of a sucrose-histidine buffer to remove extra-liposomal ammonium sulphate for dialysis after hydration of the phospholipids and liposomes are formed. Mammarella does not teach or suggest, *inter alia*, at least two elements of the claims: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution. Further, as described above, Mammarella is NOT proper prior art. However, even if it was, it would still be deficient in its asserted teachings and uncured by the remaining references for at least the following reasons.

(1) Mammarella does not teach or suggest non-pegylated liposomes

Mammarella uses a phosphatidyl ethanolamine derivatized with O-methyl-polyethylenglycol-2000 that is a pegylated phosphatide, whereas the present application is for non-pegylated liposomes. For at least this reason, one of ordinary skill would not have combined Mammarella with the applied references to yield the instant claims.

(2) Mammarella does not teach or suggest "removing ammonium sulphate from extraliposomal hydration medium by dialysis using a sucrose-histidine buffer solution"

As with all the other references, Mammarella does not teach or suggest using a sucrose-histidine buffer solution as claimed. The Examples of Mammarella clearly reveal this deficiency.

For instance, in Example 1 (paragraph [0046]), the phospholipid film is taken up in an ammonium sulphate solution. There is no sucrose in this solution. Furthermore, the Example yields liposomes without removing extra-liposomal ammonium sulphate.

Example 6 (paragraph [0066]) states: "A liposome suspension obtained as described in Example 1 is dialyzed against a solution of sucrose 10% (w/v) in order to eliminate the ammonium sulfate on the outside of the liposomes"(emphasis added). There is no histidine in this dialysis solution. AFTER dialysis (paragraph [0067]), the drug is loaded into the liposome using a sucrose-histidine buffer. Thus, Mammarella does not remove "ammonium sulphate from extraliposomal hydration medium by dialysis using a sucrose-histidine buffer solution" as required by the claims.

Moreover, the intention of Mammarella is to prepare liposome to increase drug loading efficiency, not to increase blood circulation as is accomplished in the instant invention.

For at least these reasons, one of skill in the art would not have combined the teachings of Mammarella with the other cited references and even if such combinations were made, they would still be deficient in teaching or suggesting the features of the claims.

f. Deficiencies Specific to Papahadjopoulos (US 4,235,871)

Papahadjopoulos is applied in grounds 3, 4, 5, and 7. In each of these combinations, Papahadjopoulos is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. Papahadjopoulos does not teach or suggest, *inter alia*, at least two elements of the claims: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

The Final Rejection asserts Papahadjopoulos for disclosing the claimed amount of hydration medium, specifically, "the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present" recited in claims 1 and 63. However, this reference is deficient on many fronts, including its assertion to teach the amount of hydration media.

(1) Papahadjopoulos does not teach or suggest "the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present"

In evaluating Papahadjopoulos, it is clear that the claimed amount of hydration medium is not taught. For example, a careful analysis of Examples 1 and 2 reveal this deficiency.

Calculation of hydration buffer volume per mM of lipid for Examples 1 and 2: The amount of phospholipid used is 50 μM (cholesterol is 50 μM) and there is 5ml ether in organic phase. In addition, there is 1.5 ml aqueous phase buffer plus 1 ml (– 10mg) from the alkaline phosphatase solution. Although it is not precisely clear how much water of this aqueous phase remains in viscous gel, appellants assume that the maximum is 2.5 ml (since this was the staring amount). In addition, another 1.5 ml of aqueous phase buffer is added to the gel when it changes to liposomes. At that stage the maximum water content would be 4 ml. Thus, 4 ml of aqueous phase for 50 μM converts to 4,000 per 50 mM, or 80 ml per mM, which is clearly outside of the range of the claimed invention. Even if one were to guess that there would be some loss of water during intermediate evaporation, even if assuming that the aqueous phase may be less than 4 ml (such as 3ml), there would be 3 ml of aqueous phase per 50 μM of phospholipid. This converts to 3000 ml per 50 mM; or 60 ml per mM. This is certainly outside the range of the instant invention. Even if one calculated that only 2.5 ml aqueous phase remained, it would still be

2500 ml per 50 mM or 50 ml per mM and 2000 ml per 50 mM or 40 ml per mM respectively, which is above the range given in instant invention.

(2) Papahadjopoulos does not teach or suggest removal of the solvent before hydration

The Final Rejection's statement on page 5: "The methods involve either removal of the organic solvent before hydration (Example 1) or making an emulsion using an organic solvent containing phospholipid and an aqueous medium and evaporating the organic solvent (Example 2)" is incorrect. As can be seen from reading the Papahadjopoulos patent, in Example 1 there is no removal of solvent before hydration, because the added solvent ethyl ether is present. There is no difference in Example 2 in this respect. Both examples follow the same procedure and the same quantities of the materials are used (the only difference being the type and amount of the biologically active material used). The aqueous level is the same in Examples 1 and 2. In other words, Papahadjopoulos does not teach a method of removing all solvent before hydration, to make lipid as of a dry film type before hydration, as in the instant invention. To the contrary, Papahadjopoulos needs the solvent to make an emulsion of water-in-oil when the buffer is added. Therefore, there is no teaching or suggestion in Papahadjopoulos whatsoever that solvent should be removed before adding a hydration medium.

(3) **Papahadjopoulos relates to entrapment quality and has no teaching regarding the claimed long-circulating liposomes or the hydration media**

Papahadjopoulos correlates ionic strength of buffer with the entrapment quantity of biological material: encapsulation efficiency relates to the ratio of organic phase to aqueous phase and concentration of lipid/phospholipid in the two phase system. However, it neither measures nor correlates any parameter to the increase in circulation time of the liposome prepared, and in fact, has no such study on circulation time of the liposomes. Just because it was erroneously believed that Papadopoulos has a similar ratio of aqueous phase per phospholipid, it does not mean that it is obvious to one of ordinary skill to think that this ratio will increase circulation time of liposome. It is not possible to predict what process steps give rise to longer-circulation time that results in a greater efficiency of drug delivery and (hence a greater reduction in breast tumor when the liposomes are filled with doxorubicin) and, therefore, not obvious to choose a particular ratio of aqueous phase to phospholipid from one reference for use in another process having altogether different process conditions. Therefore, it is unlikely to motivate one skilled in the art to think of applying a particular range of volume of aqueous hydration medium to the method of the present invention, which is different from that of Papahadjopoulos. In Examples 1 and 2 of Papahadjopoulos, the hydration buffer is sodium chloride/histidine/TES buffer, whereas in the instant invention, the hydration buffer is ammonium sulphate/sucrose. It is impossible to predict which process steps will produce liposomes that will give rise to longer circulation time and more efficient drug delivery and (hence a greater reduction in breast tumor when the liposomes are filled with doxorubicin). Therefore, it is incorrect to say it is obvious to choose a particular volume of hydration medium of a different composition from that used in

another process having altogether different processing conditions to get liposomes having pharmacological properties not reported or referred to, or suggested in that process.

(4) Papahadjopoulos does not teach the removal of the solvent before the addition of the aqueous phase

Since the instant invention also teaches a method wherein the solvent is removed before (or after) the addition of aqueous phase, (both of which give the same results), it is not persuasive to say that the step of removing the solvent before the addition of the aqueous phase is obvious from Papahadjopoulos. It should be noted that in Example 1 of Papahadjopoulos, one solvent is removed but another is added to dissolve the lipids, and the aqueous phase is added to the solution. Papahadjopoulos does not teach the removal of the solvent before the addition of the aqueous phase as argued by the Office Action. Papahadjopoulos' method does not say that liposomes can also be made by first removing the organic solvent and then hydrating the phospholipids. It is not obvious to think that it will work that way also.

On pages 5-6 of the Final Rejection it states that:

> Making an emulsion of the phospholipid containing organic solvent and an aqueous medium in the ratios of 1millimole of lipid/15 ml of aqueous medium and removing the organic solvent to form liposomes would have been obvious to one of ordinary skill in the art since Papahadjopoulos teaches that the liposomes can be produced by either process.

Appellants note that the drug to be encapsulated is incorporated in the aqueous phase in Papahadjopoulos procedure, whereas in the instant invention the empty liposomes are first prepared and the drug is loaded afterwards. The procedures are thus quite different and therefore it would not be obvious to one of ordinary skill in the art to think of using the step of removing

the solvent after the liposomes are formed in the process for making non-pegylated long-circulating liposomes without any drug loaded in it.

(5) Papahadjopoulos does not teach or suggest use of ammonium sulphate and sucrose as a hydration buffer

As explained above, Papahadjopoulos does not teach or suggest use of ammonium sulphate and sucrose as a hydration buffer and further the buffers are internal to the liposomes and are not used to remove extra-liposomal hydration salt, for which instant invention uses a histidine-sucrose buffer.

Papahadjopoulos discloses the preparation of liposomes by emulsifying a mixture of lipids in organic solvent and an aqueous mixture of the active material for encapsulation; and then removing the organic solvent and suspending the resultant gel in water. Sucrose is included amongst the exemplified active materials for encapsulation (*see* column 6, lines 31-43) and in the hydration buffer (*see* column 10, lines 53-56), but there is no reference to ammonium sulfate. Thus, there is no teaching of a hydration buffer comprising ammonium sulfate and sucrose.

The argument made in the office action pointing out that in quantitative aspects the instant invention and Papahadjopoulos are the same is not persuasive when they are qualitatively quite different materials: The hydration media is different, the drug is different, and the method of drug loading is different. Over and above, the quantitative aspects are also not the same as shown above. In a broad sense Papahadjopoulos method of invention calls for the formation of "inverted micelles" in an organic phase and then the removal of organic phase. The system then spontaneously reverts to a bilayer-like structure, with a large amount of aqueous phase

encapsulated in large oligolamellar vesicles. Accordingly, the methods of Papahadjopoulos and the claimed invention are not even similar.

(6) Papahadjopoulos does not teach or suggest other numerous claim elements

Examples 1 and 2 of Papahadjopoulos employ Phosphatidyl glycerol, the use of which is not indicated or claimed in the instant invention. In the instant invention the long circulation effect is achieved without the use of phosphatidyl glycerol; and therefore, the procedure of Examples 1 or 2 is not relevant to one of ordinary skill in the art to think that a particular range of ratios of aqueous phase to organic phase would lead to long circulating liposome having greater efficiency in reducing breast tumor, when loaded with doxorubicin, without the use of phosphatidyl glycerol.

In Papahadjopoulos, a buffer can be added during the liposome formation (*see* column 5, lines 30-35 & column 6, lines 5-13) and exemplified buffers include sodium chloride/histidine/2-{[tris(hydroxymethyl)methyl]amino} ethanesulfonic acid (TES) (*see* Examples 1, 2, 5, & 6). Histidine/TES buffer has been used in Examples 1 and 2 aqueous phase. There is no use of such buffer in the hydration medium in the instant invention.

These multiple deficiencies are not cured by the applied references that are applied along with Papahadjopoulos in grounds 3, 4, 5, and 7. Indeed, the references that are asserted to cure the acknowledged deficiencies of Papahadjopoulos are themselves deficient for the reasons presented above and below.

g. Deficiencies Specific To Janoff (US 4,880,635)

Janoff is applied in grounds 4, 5, and 7. In each of these combinations, Janoff is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. Janoff does not teach or suggest the hydration media claimed in the present invention. Janoff does not teach or suggest, *inter alia*, at least two elements of the claims: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

The Final Rejection on page 13 states:

> Janoff teaches that sugars such as sucrose when present both inside and outside would enable the liposomes to retain Adriamycin during dehydration and rehydration (col. 21, line 23 through col. 21 line 27). Janoff further teaches the hydration of 80 micromoles of lipid with 21 ml of buffer (25 ml per mmole).

Janoff discloses the preparation of dehydrated liposomes by drying liposome preparations under reduced pressure in the presence of one or more protective sugars. Exemplified sugars include sucrose. The dehydration is conducted under vacuum with or without prior freezing of the liposome preparation. There is no reference to any ammonium salt or to any sulfate. Reference is made to loading rehydrated liposomes using a concentration gradient created after rehydration. In the exemplified processes, 80 μmoles EPC was hydrated with 2 ml aqueous solution containing 150 mM sodium chloride, 20 mM HEPES and the respective sugar (see column 8, lines 40-63).

The fact that sucrose or other sugar inhibits leakage of an encapsulated active material from dehydrated liposomes is no indication that it will prevent leakage from the liposomes prior to dehydration or from the rehydrated liposomes after loading with the required drug or other

active material. Further, there is no evidence to say that leakage of liposomes has any relation with long-circulation of liposomes. The instant invention hydrates phospholipids to form liposomes, Janoff hydrates dehydrated liposomes. These are quite different things.

It is interesting to note that the Janoff patent was first published as a PCT 86/1103 on February 27, 1986. Subsequently Mayer filed a patent on March 5, 1987 (see WO8806442 (A1) 1988-09-07) and referred to Janoff noting that sucrose can be used for dehydration. Mayer did not think of using sucrose in a hydration medium for hydrating phospholipids in making liposomes. Thus such use was not obvious from Janoff. Even as on the date of filing the instant application, December 31, 2002 (more than 15 years after Janoff publication), nobody has used sucrose in the medium for hydration of phospholipids. This fact clearly establishes that it is not obvious to think that sucrose in combination with ammonium sulphate can be used in the phospholipid hydration medium for getting leak-proof liposomes and long-circulating non-pegylated liposomes.

In Janoff, extrusion technique vesicles (ETVs) were prepared using a solute solution containing adriamycin and 250 mM trehalose. The samples were dehydrated for 24 hours without prior freezing. The adriamycin content of the initial sample and the rehydrated vesicles was determined as described in Example 1.

As clearly demonstrated by this Example, the sugar trehalose is capable of protecting liposomes during dehydration and subsequent rehydration so that more than 90% of the material encapsulated within the liposomes is still retained therein after rehydration.

The Examiner states that Janoff teaches addition of sucrose to the phospholipid hydration HEPES buffer while preparing vesicles for protection of liposome membranes during dehydration and rehydration. The effect of such sugar addition while preparing vesicles is

performed with certain buffers. In Example 1, ETVs are used and the concluding lines (Col. 11, lines 31 – 38) are as follows:

> The results of these experiments are shown in Table 1. As shown therein, more than 90% of the drug is retained following dehydration and rehydration, i.e. the same levels as those achieved with $^{22}Na^{+}$ and ^{3}H-inulin. Moreover, the rate of leakage of adriamycin from the rehydrated vesicles is comparable to the rate observed with vesicles which have not been dehydrated (see Bally, et al., (1985), Biochim. Biophys. Acta., 812:66).

Appellants respectfully point out there is no need for dehydration or rehydration of liposomes, which is the case in the instant invention, and accordingly there is no need to add sugar according to Janoff. Janoff does not suggest that sugar addition will increase the circulation time. *See* Col.11, lines 39 – 43 noting that: "As clearly demonstrated by this Example, the sugar trehalose is capable of protecting liposomes during dehydration and subsequent rehydration so that more than 90% of the material encapsulated within the liposomes is still retained therein after rehydration." This conclusion also says that trehalose offers protection during dehydration and rehydration to materials encapsulated within the liposome and has nothing to do with increasing circulation time. It does not say that when liposomes do not go through dehydration or rehydration, trehalose will offer protection or not.

Further the results of experiments in Example 7 of Janoff leads to the conclusion:

> As shown by these results, well over 80% of the internal contents of each of the three types of liposomes were retained after the dehydration/rehydration process without the use of any protective sugars. Moreover, adding trehalose to these types of liposomes somewhat decreased, rather than increased, the amount of internal contents retained in the liposomes after the dehydration/rehydration process.

Column 16, lines 37 – 44.

This paragraph actually shows that the trehalose decreased the amount of entrapment. Thus, it is not proper to say that by reading Janoff a person having ordinary skill in the art will think that addition of sugar to ammonium sulphate containing hydration medium when there is no hydration and dehydration of liposomes, would produce liposomes having a long-circulation time.

It should be remembered that all inventions are made from known things and facts. Just knowing that sugar is a material that protects liposomes during rehydration, does not make it obvious to make liposomes with long circulation time and increase efficiency of reducing breast tumor when it is loaded with doxorubicin. All other factors including the materials used, each and every step in the preparation of such liposome contributes to make the invention. It is the concerted effect of the total process that gives the unexpected results.

Janoff does not show the addition of sucrose/sugar to the hydration media for getting it inside the liposome, and there is no motivation for doing so by reading Janoff --its abstract clearly says it is for stabilizing liposomes during hydration and rehydration.

The Examiner has asserted that Janoff teaches the use of a hydration media at 25 ml per mM of phospholipid. What is the hydration media? Sodium chloride, HEPES and trehalose buffer; what is the phospholipid? Egg phosphatidyl choline. This reference to 25ml per mM is not relevant to the conditions in instant invention – the phospholipids are different and the hydration media is different.

In column 5, line 54-68, Janoff writes:

> So that the liposomes will survive the dehydration process without losing a substantial portion of their internal contents, it is important that one or more protective sugars be available to interact with the liposome membranes and keep them intact as the water in the system is removed. A variety of sugars can be used, including

> such sugars as trehalose, maltose, sucrose, glucose, lactose, and dextran. In general, disaccharide sugars have been found to work better than monosaccharide sugars, with the disaccharide sugars trehalose and sucrose being most effective. Other more complicated sugars can also be used. For example, aminoglycosides, including streptomycin and dihydrostreptomycin, have been found to protect liposomes during dehydration (emphasis added).

This paragraph says that sucrose interacts with liposome membrane and keeps them intact as the water in the system is removed, and the liposome will survive the dehydration process. In the instant invention, there is no stage of dehydration or rehydration of the liposome; there is only hydration of phospholipids to prepare liposomes. So this quotation is irrelevant. However, upon reading the entire paragraph it is clear that trehalose or sucrose is useful for protection in dehydration, which is not a step in the instant process. The question is -- does Janoff suggest the use of sucrose with ammonium sulphate for hydration of phospholipid to form liposome? No. He does not. In Col.8, lines 40 –43 where Janoff describes how vesicles ETV are prepared, it is clear that Janoff does not use sugar with ammonium sulphate during hydration of phospholipids for making liposomes. Rather Janoff is using sodium chloride, and HEPES buffer with sugar for protecting liposome structure during dehydration. The hydration of phospholipids with ammonium sulphate and sucrose to form liposomes is for the first time shown in the present invention. The question is, is it obvious to one skilled in the art to change from sodium chloride, HEPES and sucrose buffer to ammonium sulphate and sugar as a hydration medium for hydrating phospholipid, and if so for what reason? Even Kirpotin does not suggest to one skilled in the art to change to ammonium sulphate when it shows that a polymer is much better. Papahadjopoulos does not use ammonium sulphate, so appellants assert that it can not be said

that it is obvious to use ammonium sulphate and sugar as phospholipid hydrating medium for making liposomes.

These substantial deficiencies are not cured by the applied references that are applied along with Janoff in grounds 4, 5, and 7. Indeed, the references that are asserted to cure the acknowledged deficiencies of Janoff are themselves deficient for the reasons presented above and below.

h. Deficiencies Specific To Radhakrishnan (US 5,192,528)

Radhakrishnan is applied in grounds 5, 7, and 8. In each of these combinations, Radhakrishnan is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. Radhakrishnan does not teach or suggest, *inter alia*, at least two elements of the claims: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

Radhakrishnan discloses the use of aerosolized aqueous suspensions of corticosteroid for inhalation to treat respiratory tract conditions or diseases. The exemplified processes for liposome preparation are by solvent-injection (see column 5, lines 5-15 & Example 1A) and lipid film hydration (see column 5, lines 16-29 & Examples 1B & 2). In each case, the corticosteroid is entrapped during liposome formation. There is no disclosure of the presence of ammonium sulfate or sucrose in an aqueous hydration media or of the loading of corticosteroid into a pre-formed liposome.

This deficiency is not cured by the applied references that are applied along with Radhakrishnan in grounds 5, 7, and 8 Indeed, the references that are asserted to cure the

acknowledged deficiencies of Radhakrishnan are themselves deficient for the reasons presented above and below.

i. Deficiencies Specific To Uchiyama (121 Int'l J. of Pharmaceutics 195-203 (1995)

Like Radhakrishnan, Uchiyama is also applied in grounds 5, 7, and 8. In each of these combinations, Uchiyama is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. Uchiyama does not teach or suggest, *inter alia*, at least two elements of the claims: 1) an aqueous hydration medium comprising ammonium sulfate and sucrose; and 2) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

Uchiyama reports the effect of size and fluidity of liposomes on their accumulation in tumors. The liposomes were prepared from EPC or hydrogenated EPC (HEPC), dicetyl phosphate (DCP) and cholesterol (CHOL) in the molar ratio 5:1:4 by hydration with an isotonic phosphate buffer (not ammonium sulfate and sucrose as required by the present invention). *See* page 196-197. It was concluded that accumulation of liposome from the vascular space into a tumor is primarily governed by their size and not by their membrane fluidity or blood circulation time.

The Examiner's calculation of the amount of aqueous hydration media per mmole phospholipid from Uchiyama (incorrectly) assumes that all lipids are phospholipids. However, 40 mole percent of the lipids is provided by cholesterol (CHOL). Accordingly, the 5 ml phosphate buffer was added to 200 μmole lipid of which 60% was provided by the phospholipids

(EPC/HEPC & DCP) and hence the relative amount of hydration media is 41.5 ml per mmole phospholipid. This is outside the range required by the claims.

This deficiency is not cured by the applied references that are applied along with Uchiyama in grounds 5, 7, and 8. Indeed, the references that are asserted to cure the acknowledged deficiencies of Uchiyama are themselves deficient for the reasons presented above and below.

C. CONCLUSION

Thus, reviewing all of the cited references used in various combinations for the 35 U.S.C. §103(a) rejections, clearly shows that: (1) the cited references do not actually teach or suggest the elements the Examiner alleges and, in some cases, the references actually teach away from using the recited element; (2) the Examiner has appeared to pick and choose certain words from the cited references to argue they teach an element of the claim, and in so doing, has therefore ignored how the allegedly-taught element has actually been used in the reference; (3) not all of the claimed elements are taught or suggested even by all NINE of the cited references. Appellants have attached a claim chart at §IX at Appendix 2 that summarizes the alleged teachings of the cited art against the elements of claim 1. This chart summarizes the arguments above and clearly shows that combining all of these references, there still is a failure to teach or suggest each and every claim element. Accordingly, Appellants request withdrawal of all the grounds of rejection for all of the pending claims.

For at least the above-described and substantial failures of the references both separately and combined, the instant claims are in condition for allowance. In view of the long prosecution

history, and the clear errors in application of the standards for patentability, Appellants respectfully request a direct allowance of the claims.

The requisite fee due upon filing of this brief is submitted herewith. Any additional fee is to be charged to Baker Donelson Bearman Caldwell & Berkowitz, PC, Deposit Account No. 50-4254, referencing docket number 2912919-020000.

Respectfully submitted,

BAKER, DONELSON, BEARMAN, CALDWELL & BERKOWITZ, P.C.

DATED: July 1, 2010

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VIII. Claims Appendix

1. (Rejected) A process for manufacture of long circulating non-pegylated liposomes comprising:

dissolving one or more phospholipids and one or more sterols in a solvent or mixture of solvents;

wherein the one or more phospholipids is a saturated phosphatidylcholine selected from the group consisting of distearoyl phosphatidylcholine (DSPC), hydrogenated soya phosphatidyl-choline (HSPC) and mixtures thereof;

removing the solvent or mixture of solvents and adding an aqueous hydration media to the phospholipids and sterols; or adding an aqueous hydration media to the phospholipids and sterols in the solution; and removing the solvent or mixture of solvents;

wherein the aqueous hydration media comprises ammonium sulfate and sucrose and the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present to form long circulating non-pegylated liposomes; and

removing ammonium sulphate from extraliposomal hydration medium by dialysis using a sucrose-histidine buffer solution.

2. (Rejected) The process of claim 1 wherein the amount of aqueous hydration media used is 30 ml for each mmole of phospholipid in the lipid solution.

3. (Rejected) The process of manufacture of non-pegylated liposomes of claim 1 further comprising loading the liposomes with a therapeutic or diagnostic agent after removal of the ammonium sulphate from the extraliposomal hydration medium.

4. (Rejected) The process of claim 3, wherein the therapeutic agent is an antineoplastic agent.

5. (Rejected) The process of claim 4, wherein the antineoplastic agent is selected from the group consisting of Doxorubicin hydrochloride, Daunorubicin hydrochloride, and Epirubicin hydrochloride.

6. (Rejected) The process of claim 5, wherein the antineoplastic agent is Doxorubicin hydrochloride.

7. (Rejected) The process of claim 1, wherein the molar ratio of phospholipid to sterol is from about 1:0.1-1:2.

8. (Rejected) The process of claim 7, wherein the molar ratio of phospholipid to sterol is about 1:0.7.

9. (Canceled)

10. (Rejected) The process of claim 1, wherein the concentration of ammonium sulfate in aqueous hydration media is not less than 125 mmoles/liter.

11. (Canceled)

12. (Rejected) The process of claim 1, wherein the phospholipid has a minimum of sixteen carbons fatty acid chain.

13. (Canceled)

14. (Rejected) The process of claim 1, wherein the phospholipid is distearoyl phosphatidylcholine (DSPC) and wherein the sterol is cholesterol.

15. (Rejected) The process of claim 1, wherein the non-pegylated liposomes are successively extruded through series of filters having pore sizes from 0.4 μm to 0.05 μm for sizing.

16. (Rejected) A liposome manufactured by the process of claim 1.

17. (Rejected) The liposome of claim 16, wherein the phospholipid comprises distearoyl phosphatidylcholine (DSPC) and the sterol comprises cholesterol.

18. (Rejected) The liposome of claim 16, wherein the non-pegylated liposome further comprises a therapeutic or diagnostic agent.

19. (Rejected) The liposome of claim 18, wherein said therapeutic agent comprises an antineoplastic agent.

20. (Rejected) The liposome of claim 19, wherein the antineoplastic agent is selected from the group consisting of Doxorubicin hydrochloride, Daunorubicin hydrochloride, and Epirubicin hydrochloride.

21. (Rejected) The liposome of claim 20, wherein the antineoplastic agent is Doxorubicin hydrochloride.

22. (Rejected) The liposome of claim 16, wherein the average size of liposome is 0.06 μm to 0.16 μm in diameter.

23-62. (Canceled)

63. (Rejected) A process for manufacture of non-pegylated liposomes comprising:

forming a lipid film by evaporating a solvent from a lipid solution comprising one or more phospholipids, a sterol, and a solvent; and

hydrating the lipid film by adding an aqueous hydration media to form a non-pegylated liposomal composition; wherein the aqueous hydration media comprises ammonium sulfate and sucrose and wherein the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present in the lipid solution; and

removing ammonium sulphate from extraliposomal hydration medium using a sucrose-histidine buffer solution.

64. (Rejected) The process of Claim 63 wherein the aqueous hydration media comprises greater than 125 mM ammonium sulfate and 100 mM to 500mM sucrose.

65. (Rejected) The process of Claim 63 wherein the aqueous hydration media comprises greater than 125 mM ammonium sulfate and 250 mM to 300 mM sucrose.

66. (Rejected) The process of Claim 63 wherein the amount of histidine in the sucrose-histidine buffer is 1 mM to 100 mM.

67. (Rejected) The process of Claim 63 wherein amount of histidine in the sucrose-histidine buffer is 8 to 12 mM.

68. (Rejected) The process of Claim 63 wherein amount of histidine in the sucrose-histidine buffer is 10 mM.

69. (Rejected) The process of Claim 1 wherein the long circulating non-pegylated liposomes have a blood circulation half life of at least 25 times longer than conventional non-liposomal formulations when tested in Swiss albino mice at equivalent doses.

IX. Evidence Appendix

Contents:

Appendix 1:

Declaration by Pai Srikanth Annappa

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Daftary, et al.

Confirmation No.: 6940

Serial No.: 10/748,094

Examiner: Kishore, G.

Filed: December 31, 2003

For: Non-Pegylated Long-CirculatingLiposomes

DECLARATION UNDER 37 CFR §1.132

I, Pai Srikanth Annappa, do hereby declare and say as follows:

1. I have a masters degree in pharmacy from College of Pharmaceutical Sciences, Manipal, India. I currently hold the position of Senior Vice-President of Research and Development (Drug Delivery) for Bharat Serums and Vaccines Ltd.

2. I am a co-inventor on the following issued patents:
   a. Parenteral Cisplatin Emulsion (WO2001/007058)
   b. Clear Propofol Compositions (WO2002/45709)
   c. Amphotericin B Aqueous Composition (WO2002/069983)
   d. Amphotericin B Structured Emulsion (WO2000/197778)
   e. Clear Aqueous Anaesthetic Composition (WO2001/97796)
   f. Liquid Stable Composition of Oxazaphosphorine with Mesna (WO2004/022699)

g. Ifosfamide Compositions for Parenteral Administration and a Process for their Preparation (WO2004/050012)

h. Non-pegylated Long-circulating Liposomes (WO2004/058140)

i. Stable Emulsion Compositions for Intravenous Administration having Preservative Efficacy (WO2006/030450)

j. Intravenous Propofol Emulsion Compositions having Preservative Efficacy (WO2007/052288)

k. Aqueous Anaesthetic Compositions Comprising Propofol (WO2007/052295)

l. Propofol Emulsion Compositions for Intravenous Administration (PCT Appl.No. PCT/IN06/000466)

3. I have worked in the areas of developing parenteral products comprising liposomes, microspheres, intravenous emulsions/suspensions, phospholipid complexes, and soluble drug complexes.

4. I have reviewed and am familiar with the contents of the above-referenced patent application ("the present application") of which I am a co-inventor. I have also read the Office Action dated July 23, 2007 and the references cited therein. In particular, I have read U.S. Patent 6,110,491 (issued to Kirpotin and hereinafter referred to as "Kirpotin").

5. In the Office Action, the Examiner contends it is obvious to one of ordinary skill in the art to vary the amounts of the hydrating medium to obtain the best possible results. I respectfully disagree with this conclusion and my reasons are set forth below in the instant Declaration.

6. In summary, Kirpotin does not teach or suggest each and every element in the claimed invention. The novel liposomes of the present invention are made without polyethylene glycol (PEG), and the novel process of manufacture involves using a lower amount of hydration buffer than what was previously used to provide stable liposomes. Kirpotin does not teach non-pegylated liposomes nor does this reference

teach making the liposomes with a lower amount of hydration media. In addition, no one would even be motivated to make the present invention by reading Kirpotin, alone or in combination with the cited references, as the problems addressed by Kirpotin are different from the problems addressed by the present invention. The present invention concerns the manufacture of stable liposomes without using PEG (by using a smaller amount of hydration buffer) whereas Kirpotin tries to address issues surrounding liposome loading.

7. Kirpotin simply does not teach or suggest a process for the manufacture of long circulating non-pegylated liposomes as set forth in claim 1. In fact, Kirpotin teaches the preparation of liposomes containing polyethylene glycol (*i.e.* derivatized distearolphosphatidyl ethanolamine (PEG-DSPE)) to increase liposome stability (*see* Kirpotin's Examples 1 and 3-8).

8. Kirpotin also fails to teach or suggest an "aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present in the lipid solution" as recited in claim 1 of the present application. Based on my reading of Kirpotin, there is absolutely no teaching or suggestion to remove PEG and manufacture liposomes with a surprisingly low amount of hydration media. Further, it is my opinion that no one would one have thought to use such low amounts of hydration media to achieve a long lasting non-pegylated liposome upon reading Kirpotin (alone or in combination with the other cited references).

9. Furthermore, it was unexpected that by simply reducing the amount of hydration buffer, one would obtain a stable liposome without the need for PEG. The volume of aqueous hydration media as recited in claim 1 is less than what was previously known or considered an acceptable amount of aqueous hydration media. Thus, the volume of the aqueous hydration media as recited in claim 1 demonstrates a controlled reduction as compared to the amounts of hydration media used in conventional liposome and pegylated liposome manufacture. Surprisingly, we found that by reducing the volume of aqueous hydration media, and the composition of the

hydration media used, the toxicity of the doxorubicin loaded liposomes was reduced. Not bound by theory, we believe that the phospholipids were able to pack tighter together resulting in a thicker liposome membrane or "shell." The thicker "shell" provided for increased stability, long-circulation and slow release. The increased stability resulted in decreased toxicity without the need for PEG. The LD_{50} values of the different Doxorubicin compositions studied are provided Table 1 of page 7 of the published application. The LD_{50} dose was found to be 16.13 mg/kg whereas the LD_{50} dose for the marketed conventional preparation (ADRIAMYCIN) was 10.29 mg/kg. The LD_{50} for the marketed pegylated liposomal preparation CAELYX was 13.5 mg/kg. These results show that non-pegylated liposomes of the present invention have a reduced toxicity as compared to other Doxorubicin formulations and to pegylated-liposomal Doxorubicin formulations, and would be further devoid of hand foot syndrome associated with pegylation.

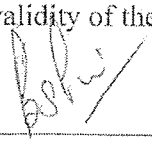
10. In my opinion, no one would be motivated to even consider Kirpotin for suggesting a nonpegylated liposome made by reducing the amount of hydration buffer as Kirpotin attempts to provide a solution to an entirely different problem from the problem addressed by the present invention. Kirpotin teaches methods of producing pegylated (PEG) liposomes in an effort to increase encapsulation efficiency. In other words, Kirpotin is concerned with methods of increasing the loading of the liposomes. Thus, the invention taught by Kirpotin and the present claimed invention are aimed at solving two entirely different problems.

11. In contrast to Kirpotin, the present invention is concerned with making liposomes without the use of PEG. The present invention is directed to non-pegylated liposomes and methods of their manufacture to avoid the occurrence of "Hand-Foot syndrome," associated with preparations containing pegylated phospholipids. *See* present published application, para. 0013. Additional advantages in avoiding pegylation are also illustrated in Table 1 on page 7 of the published application. Table 1 shows that non-pegylated doxorubicin liposomes of the present invention have lower toxicity than conventional pegylated liposomes.

12. Furthermore, the non-pegylated liposomes of the present invention have been shown to be more effective in reducing tumor weight in comparison to pegylated liposomes. The test results summarized in Table 2 support this position. Specifically, the difference in tumor weight and effectiveness was measured by T/C % (test to control percentage). In this study (Example VI), the highest ratio of T/C using CAELYX was -78 at 12 mg/kg and -34.7 at 6 mg/kg, whereas when using the non-pegylated doxorubicin liposomes of the present invention, the highest was -93.4 at 12 mg/kg and -89.43 at 6 mg/kg. These results demonstrate that the non-pegylated doxorubicin liposomal compositions of the present invention are more effective in reducing tumor weight than the currently marketed pegylated liposomal formulation.

13. In conclusion, it is my opinion that the present invention would not be obvious light of Kirpotin, alone, or in combination of the cited references.

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Pai Srikanth Annappa

Date: October 18, 2007

Appendix 2: Claim Chart

10/748,094 (instant application)	Long-circulating NON-PEGYLATED LIPOSOME	Aqueous hydration media comprises ammonium sulfate and sucrose	10-35 ml for each mmole of phospholipid	Removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution
Kirpotin	No-Just PEG-liposomes And method of LOADING liposomes	No-Teaches away: as ammonium sulfate had lowest performance for PEG liposomes	No	No-No mention of sucrose-histidine buffer
Forssen	No-Teaches a method of making liposomes with the vinca alkaloid for purpose of retaining contents	No-Buffer with EITHER ammonium salt of one of the counter-ions OR 300mM sucrose	No	No
Janoff	No	No-Mentions sugars IN the bilayer for the purpose of protective the internal contents during dehydration steps not FOR hydration	No	No
Papahadjopoulos	No	No	No	No-Mentions histidine buffer in hydration buffer step NOT a histidine sucrose solution for removal of extraliposomal ammonium sulphate salt
Hong	No-PEG liposomes disclosed	No-No mention of sucrose	No	No
Radhakrishnan	No	No	Yes-but phosphate buffered saline 50ml for 2mM of the lipid mixture	No
Uchiyama	Yes-but long-circulation of HEPC liposomes	No-Isotonic phosphate buffer used	No-45 mL per mmole of phospholipid	No
Mammarella*	No-PEG liposomes Contains lysophopholipid	No-Only ammonium sulphate	No	No-Sucrose for removal of ammonium ions
Wong*	No-PEG liposomes	No-Only ammonium sulphate	No	No-Sucrose for removal of ammonium sulfate

*post-dated reference

X. Related Proceedings Appendix

There are no proceedings related to the instant proceeding.